

**Title** : SELECTIVE INHIBITION OF THE AROMATIC HYDROXYLATION OF BUPIVACAINE BY HALOTHANE

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**Introduction:** Patients for whom regional anesthesia is the primary technique often require general anesthesia supplementation during the operative period. Recently, diethyl ether has been reported to suppress oxidative and glucuronic acid conjugation pathways in isolated rat hepatocytes using antipyrine and paracetamol as study drugs.(1) In 1971, Brown reported the suppression of N-dealkalation and enhancement of the hydroxylation pathways of drugs in the presence of 2 and 6% halothane using liver microsomes as the model(2). The present study was undertaken to examine the possibility that halothane could suppress oxidative and conjugative metabolic pathways critical to the detoxification of local anesthetics, using bupivacaine as a model local anesthetic and rat hepatocyte cultures as the model system.

**Methods:** Hepatocytes were isolated from Fisher 344 rats by modification of the collagen perfusion method of Seglen(3). Following the isolation procedure, the hepatocytes were suspended at a concentration of  $1.1 \times 10^6$  cells/ml in RPMI-1640 culture media, and viabilities determined by Trypan blue exclusion tests. A minimum viability of 75% was considered acceptable. Cytochrome P-450 measurements were done on composite hepatocyte suspensions using a Beckman DU-8 spectrophotometer(4). Type I or II binding characteristics for bupivacaine and PPX were determined by measuring difference spectra using a Beckman DU-8 spectrophotometer. Hepatocytes were incubated at 37° in air, air + 0.5% halothane, air + 1% halothane, and air + 2% halothane for 10 minutes. A fixed concentration of 0.056 molar bupivacaine was introduced. This concentration was chosen, since earlier Km and Vmax studies demonstrated complete disappearance of bupivacaine over a 2-hour period. In addition, over the 2-hour time course at this bupivacaine concentration, one is able to observe the peak 2,6-pipercoloxylidide(PPX) concentration as well as the initial rate of subsequent PPX metabolism. 1-ml samples were withdrawn at 0.5, 1, 1.5, and 2 hours. Initial studies indicated that viability and metabolic integrity of the hepatocyte cultures were maintained over this time frame. Metabolism was terminated by heating each sample to 60°C. Following centrifugation, a sample of the supernatant was analyzed for bupivacaine and PPX concentrations, using gas chromatography. Halothane concentrations were verified using gas chromatography. Rates of bupivacaine disappearance were estimated from semilogarithmic plots of concentration vs. time. Estimates of initial velocities for PPX formation were obtained from concentration differences between the 10-and 20-minute point and the 20-and 30-minute points. Statistical analysis was accomplished using a student's t test.  $p < .05$  were considered significant.

Table 1: Rates of Bupivacaine Metabolism and Formation of PPX as a Function of Halothane Concentration (as a percent of control)

| Treatment            | Rate Bupivacaine Metabolism | PPX Formation   |
|----------------------|-----------------------------|-----------------|
| Control(Air)         | $1.0 \pm 0.05$              | $1.0 \pm 0.05$  |
| Air + 0.5% Halothane | $0.83 \pm 0.05^b$           | $1.11 \pm 0.05$ |
| Air + 1.0% Halothane | $0.63 \pm 0.08^c$           | $1.01 \pm 0.05$ |
| Air + 2.0% Halothane | $0.38 \pm 0.12^d$           | $0.93 \pm 0.05$ |

<sup>a</sup> Mean  $\pm$  sem; <sup>b</sup>  $p < .025$  compared to control; <sup>c</sup>  $p < .005$  compared to control; <sup>d</sup>  $p < .001$  compared to control.

**Results:** As in control studies, all reactions were first order after 30 minutes. Halothane significantly inhibits bupivacaine metabolism in a dose-related fashion over all concentration ranges studied. The initial rates of PPX formation (N-dealkalation of bupivacaine) are not significantly suppressed by halothane (Table 1). PPX remained at its peak concentration for a significantly longer time in the presence of halothane when compared to control groups. Reversal of the order of exposure of the hepatocytes to halothane and bupivacaine produced identical results. The different spectra for both bupivacaine and PPX demonstrated type I substrate binding.

**Discussion:** The mean value of 37% suppression at 2% halothane are in reasonable agreement with the findings of Aune et al.(1) N-dealkalation of bupivacaine was not significantly suppressed by halothane. These data, are in direct contrast to the study of Brown, who observed suppression N-dealkalation with type I substrates and enhanced aromatic oxidation with type II substrates. A prolonged time at the peak concentration for PPX in the presence of halothane could be due to a decrease in the rate of aromatic oxidation, which the principal route of PPX metabolism. These data suggest that patients receiving both bupivacaine and halothane may experience a decrease in bupivacaine clearance. The clinical significance of this drug interaction deserves further consideration.

#### References:

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